SOME PRELIMINARY OBSERVATIONS ON THE COMPETITIVE COLONIZATION OF FILTER-PAPER BY CELLULOSE-DECOMPOSING FUNGI

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ABSTRACT - The main objective of the present work has been to study the impact of growth-rate and cellulolytic ability on the competition between cellulose-decomposing fungi during colonization of cellulosic materials. For this reason, species of different growth-rates and of different cellulolytic abilities have been grown opposite each other in pairs and triads on filter paper. According to their competitive growth, cellulose-decomposing fungi were classified into 3 groups namely, **strong, moderate,** and **weak** competitors. It is suggested that members of the first 2 groups are those which might actively participate in the break down of cellulosic materials in the soil. The results also revealed that fungi of similar cellulolytic abilities or growth rates do not necessarily have equal competitive potentialities.

RÉSUMÉ - L'objectif principal de ce travail a été d'étudier l'influence de la vitesse de croissance et de l'activité cellulolytique dans la compétition des champignons décomposant les substrats cellulosiques. Des espèces à taux de croissance et activité cellulolytique différents ont été confrontées par deux ou trois, sur du papier filtre. Selon leur efficacité dans la colonisation, les champignons ont été classés en **forts, modérés** et **faibles** compétiteurs. On suggère que les membres des deux premiers groupes sont ceux qui participent activement à la décomposition des matériaux cellulosiques dans le sol. Les résultats révèlent aussi que les champignons d'activité cellulolytique ou de taux de croissance semblables n'ont pas forcément les mêmes potentialités dans la compétition.

MOTS CLÉS : cellulose-decomposing fungi, competition.

INTRODUCTION

In a previous study (Moustafa & Sharkas, 1972) species potentially able to colonize filter paper cellulose were isolated, from the tidal mud-flats of Kuwait, and their cellulolytic activities were tested. It was noticed that there is no coincidence between the frequency of occurrence of a particular species, on the isolation plates, and its cellulolytic activity in pure culture i.e. high frequency fungi on the filter paper are not necessarily very active cellulose decomposers while less frequent species might be.

For cellulose decomposing fungi, it is most probable that their competitive saprophytic ability rather than their cellulolytic activity determine their contribution to the process of cellulose decomposition in the soil. Also, the success of any fungus in competitive colonization is known to depend on 3 factors: its competitive ability, its inoculum potential, and environmental conditions, both biotic and abiotic (Griffin, 1972). The attributes contributing to the competitive saprophytic ability of a fungus (Garret, 1956) are: its growth rate, enzyme production, and production or tolerance of inhibitors produced by its competitors.

The present investigation was initiated therefore to study the ability of cellulose-decomposing species to colonize filter paper cellulose under competitive conditions in order to:

- ascertain the importance of both cellulolytic activity and growth-rate in the process of cellulose decomposition.

- seek an answer to a fundamental question: do fungi of equal cellulolytic activities and/or growth-rates also possess equal competitive saprophytic abilities?

- assess the reliability of using cellulose-decomposing ability, in pure culture, as a parameter to express fungal activity in the soil.

MATERIALS AND METHODS

Forty-four species, potentiality able to utilize cellulose, were grown (in triplicates) at 27°C on Whatman N° 1 filter paper to differentiate between their cellulolytic abilities and growth-rates. Czapek agar medium (without sugar) supplemented with 0.5% yeast extract was used. Linear-growth rate (Trinci, 1971; Garrett, 1980) as a growth criterion has been adopted. The daily growth rate of all species, inoculated singly, was followed over a period of 10 days thereafter final colonies diameters were measured and compared.

Delineation of fast and slow-growing species:

According to the daily rate of growth, fungi showing growth rate of 1cm or more per day were considered **"fast growers"**. Others showing growth of less than 1cm per day are regarded as **"slow growers"**.

Delineation of high and low cellulolytic ability species:

Dry weight loss (Garrett, 1983) was adopted. Fungi were grown singly on damp filter paper (soaked in mineral solution) for a period of 3 weeks at 27°C. To keep humidity inside plates as constant as possible, a few drops of mineral solution were added every 2 or 3 days. Fungi which caused a loss of 10% or more in the dry weight of filter paper were considered **"high cellulolytic species"**, while those showing a loss in dry weight less than 10% were regarded as **"low cellulolytic species"**.

Interaction between fungi in mixed cultures:

Species of different cellulolytic abilities and others of different growth-rates were inoculated opposite each other (1cm apart) in pairs and triads in order to study the eventual interaction between soil fungi during competitive colonization of cellulosic materials. For every species, 30 tests have been carried out, 15 pairs and 15 triads. The species used in these tests have been selected to fulfil the following cases of interactions:

a) Interaction between fungi of similar and different cellulolytic abilities i.e.: high x high, high x low, low x low.

b) Interaction between fungi of similar and different growth-rates, i.e.: fast x fast, fast x slow, slow x slow.

RESULTS AND DISCUSSION

The fungi studied in the present investigation are listed in Table 1, where they have been arranged, according to their growth rates, in 2 groups namely, **fast** and **slow** growing. **"Fast-growing group"** comprised 20 species showing varying degrees of cellulolytic ability. Only 7 species are highly cellulolytic. The first two members of this group namely, *Trichoderma koningii* and *Lasiobolidium orbiculoides* are extremely fast i.e. quickly covering the whole plates within 2 or 3 days. *L. orbiculoides* is a well known dung fungus (Malloch & Benny, 1973) recently recorded several times from the desert soils of Kuwait (Moustafa & Sharkas, 1982; Moustafa & Khosrawi, 1983).

"Slow-growing group" contained 24 species, of which only 8 are highly cellulolytic while 16 are low. The last four species of this group namely, *Alternaria alternata, Cladosporium cladosporioides, Scopulariopsis brevicaulis* and *Ulocladium consortiale* are very slow growers i.e. their colonies remain restricted in size and never exceed 1 or 2cm after 10 days.

Effect of cellulolytic-ability and growth-rate on the interaction between fungi:

The results of interaction studies between fungi of different growth rates and others of different cellulolytic abilities suggest that the potential for competition is not fully dependant upon these 2 factors. It was evident that high cellulolytic ability or fast growth rate of some fungal species does not imply that these species are strong competitors as might be expected. Species such as *Myrothecium verrucaria, Stachybotrys atra, Chaetomium globosum, Graphium penicillioides,* through active cellulose decomposers, are weak competitors. On the contrary, *A. flavus, A. egyptiacus, A. nidulans, P. cyclopium, P. funiculosum* proved to be good competitors though all are weak cellulose decomposers.

Table I - Cellulose decomposing fungi arranged according to their growth-rates and cellulolytic abilities*

Tableau I - Classement des champignons selon leur vitesse de croissance et leur activité cellulolytique*

Fast-growing species	Cellulolytic ability
Trichoderma koningii Oudem.	18.5
Botryotrichum piluliferum Sacc. & March	16.4
Graphium penicillioides Corda	15.2
Fusarium oxysporum Schl. ex Fr.	13.6
Chaetomium brasiliense Batista & Mont.	12.8
Lasiobolidium orbiculoides Mall. & Benny	11.6
Corynascus sepedonium (Emmons) V. Arx	10.2
Paecilomyces variotii Bain.	4.2
Aspergillus tamarii Kita	4.1
Penicillium funiculosum Thom	3.6
P. cyclopium Westling	3.3
A. quadrilineatus Thom & Raper	3.1
A. flavus Link ex Fr.	2.8
A. fumigatus Fres.	2.4
A. niger Van Tieghem	1.8
Narasinhella hyalinospora (Kuehn et al.) V. Arx	1.5
Rhizopus arrhizus Fischer	0.6
Cunninghamella phaeospora Boed.	0.4
Syncephalastrum verruculosum Misra	0.3
Actinomucor elegans (Eidam.) Benj. & Hessel.	0.3
Slow-growing species	**Cellulolytic ability**
Myrothecium verrucaria Ditmar ex Fr.	16.4
Stachybotrys atra Corda	15.6
Chaetomium cochlioides Palliser	14.8
C. globosum Kunze ex Fr.	13.2
C. olivaceum Cooke & Ellis	11.8
C. virginicum Ames	11.4
C. gracile Udagawa	11.2
C. rectopilium Freg. & Amelung	10.3
Ascotricha bosei D. Hawksw.	5.8
Phoma fimeti Brunaud	4.2
Arachniotus dankaliensis (Cast.) V. Beyma	3.4
Aspergillus ustus (Bain.) Thom & Church	3.2
A. egyptiacus Moub. & Moustafa	3.0
A. nidulans (Eidam) Wint.	2.7
A. terreus Thom	2.2
A. sydowii (Bain. & Sart.) Thom & Church	1.7
Drechslera spicifera (Bain.) V. Arx	1.4
A. ochraceus Wilhelm	1.2
A. unguis (Emile Weil & Gau.) Thom & Raper	0.9
Alternaria chlamydospora Mouchacca	0.6
A. alternata (Fr.) Keissler	0.4
Cladosporium cladosporioides (Fres.) de Vries	0.4
Scopulariopsis brevicaulis (Sacc.) Bain.	0.3
Ulocladium consortiale (Thüm.) Simmons	0.2

* For growth-rate and cellulolytic abilities see Materials and Methods.

The interaction between species, when grown opposite each other in pairs or triads, varied markedly from one case to another. The following observations are the most important:

1- On the whole, interactions between pairs of species seemed to be very specific i.e. in many cases, a particular species A may completely overgrow species B, however, it may completely fail with species C. For instance, *Lasiobolodium orbiculoides* dominated over its opponents in all pair tests but was completely overgrown by *Trichoderma koningii*.

2- In triad tests, on the other hand, the interactions are less specific. Complete overgrowth of any fungus over its two associates was never reported. In all cases species A either partly dominate over or co-exist with species B and C, e.g. *Botryotrichum piluliferum* partly dominated over *Graphium penicillioides* and *Corynascus sepedonium* but co-existed fairly well with *Fusarium oxysporum* and *Chaetomium rectopilium*. The presence of a third organism sharing the association most probably reduce the deleterious effect resulting from specific interaction. Such buffering action created by fungi growing in populations is a factor of great ecological significance contributing to the regulation of biological equilibrium in the soil. Therefore, a group of species (more than 3) in mixed culture would certainly be better and more realistic in competitive studies to avoid specific interactions, a phenomenon which does not exist in the soil. However, a technique whereby a population of several organisms can be introduced into the substrate at a time is currently not available.

3- Species belonging to one genus do not necessarily possess similar potentialities. This was prominant in species of *Chaetomium* and *Aspergillus*. Seven species of *Chaetomium* were tested but the most competitive were only *C. brasiliense*, *C. virginicum*, *C. cochlioides*. Also, *Aspergillus* is represented by 12 species but the best competitors were *A. flavus*, *A. egyptiacus*, *A. nidulans*, and *A. quadrilineatus* although all are not highly cellulolytic.

4- *Trichoderma koningii* proved to be the most powerful fungus among all species tested. It completely overgrew its opponents in the majority of pair and triad tests. Its growth however, was partly inhibited in few cases, namely when grown in triad associations with any of the following species: *A. fumigatus*, *A. flavus*, *Fusarium oxysporum* and *Syncephalastrum verruculosum*.

5- The results clearly showed that not all fast-growing and/or high cellulolytic ability species are good competitors, on the contrary many fungi of low cellulolytic ability and/or slow growth-rate are able to show good competition. Such observation many point to a fact that there must be some factors beside cellulolytic activity and growth-rate that enables these fungi to complete successfully and develop their colonies in the presence of strong cellulose decomposers. Most probably such species are able either to secrete and/or tolerate the inhibitory effect of metabolites excreted by their associates. It is likely that both factors may have little influence upon competition between cellulose-decomposers. Each of these factors may be involved rather as a privilege than a limiting factor during competition. This agrees well with the findings of Tribe (1966) who concluded that "cellulolytic

activity is secondary to other competitive characters". A further, evidence was given by Garrett (1983) during a study of filter paper decomposition by different cellulolytic species. He noticed that growth rate in some species came in the reverse order to their cellulolytic activity.

6- According to the behaviour of species when grown in pairs and triads, several growth patterns have been recorded according to which cellulose decomposing fungi could be "tentatively" classified into 3 groups as follows (Table 2):

Table 2: Cellulose decomposing fungi classified into groups and arranged in decreasing order of dominance (*) according to their interactions in pairs and triads.

Tableau 2 - Classement des champignons en 3 groupes selon les résultats des confrontations (à 2 ou 3) et arrangement en ordre décroissant de dominance*.

Group "A"		Group "B"	
Species	% dominance	Species	% dominance
Trichoderma koningii	100	*Fusarium oxysporum*	46
Chaetomium brasiliense	86	*Chaetomium rectopilium*	43
Lasiobolidium orbiculoides	73	*Corynascus sepedonium*	43
Botryotrichum piluliferum	63	*Graphium penicillioides*	40
Aspergillus flavus	53	*Chaetomium virginicum*	40
		C. cochlioides	40
		Aspergillus egyptiacus	40
		A. nidulans	36
		A.quadrilineatus	33
		Penicillium cyclopium	30
		P. funiculosum	26

Group "C"			
Species	% dominance	Species	% dominance
Chaetomium globosum	23	*A. niger*	6
C. olivaceum	22	*A. ochraceus*	6
C. gracile	20	*A. sydowii*	5
Arachniotus dankaliensis	16	*A. ustus*	4
Stachybotrys atra	13	*A. tamarii*	4
Myrothecium verrucaria	13	*Narasinhella hyalinospora*	3
Phoma fimeti	10	*Cunninghamella phaeospora*	3
Aspergillus unguis	10	*Actinomucor elegans*	3
Paecilomyces variotii	10	*Ascotricha bosei*	3
Syncephalastrum verruculosum	10	*Cladosporium cladosporioides*	2
Rhizopus arrhizus	7	*Ulocladium consortiale*	2
Scopulariopsis brevicaulis	7	*Alternaria chlamydospora*	2
Aspergillus terreus	7	*A. alternata*	2
A. fumigatus	7	*Drechslera spicifera*	2

* For % dominance see text (Materials & Methods)

Group A, Strong competitors: assigned to this group species which completely predominated over their associates in 50% or more of cases. Only 5 species belong to this group. Except for *A. flavus* all members are highly cellulolytic and fast growers.

Group B, Moderate competitors: consists of species which showed predominance or co-existence (in approximately equal colonies) with others in 25% - 50% of cases. This group comparses 11 species of various characters i.e. fast and slow growth rates, high and low cellulolytic abilities.

Group C, Weak competitors: includes species which showed just existence in the form of small restricted colonies in less than 25% of cases. Most fungi tested (28 out of 44) belong to this group.

With regard to this categorization it has to be expected that members of Groups A and B are those which participate actively in the breakdown of cellulosic materials in the soil much more than members of Group C can do although the latter group constitutes species of well known cellulolytic activity like *Stachybotrys, Myrothecium* and some species of *Chaetomium*. These species however failed to exercise their cellulolytic activity under competitive condition. For this reason, the use of cellulose decomposing ability of a species, in pure culture, as a parameter to express it activity in the soil is not reliable.

As cellulose-decomposing fungi differ in their competitive abilities, they possibly follow a sort of autonomic succession during cellulose colonization i.e. some species may anticipate others. The first wave is expected to comprise those species which possess fast growth rate. It is possible that members of this wave have no or little ability to produce or to tolerate metabolites. Therefore, growth rate here is an advantageous factor enabling such species to anticipate others during colonization in order to escape severe competition. The second wave consists of fungi which are able to interfere with others and overgrow them depending mostly upon their ability to produce and/or to tolerate the effect of metabolites. A similar pattern of succession has been reached by Tribe (1960) in his follow-up of succession of fungi during colonization of cellulose film in various Canadian soils, and by Park (1959) during a study of colonization of grass leaves and clover stolens.

There is no doubt that using unnatural substrates like filter paper in this study and others (Garrett, 1956; Park, 1975, 1976 a,b; Forbes & Dickinson, 1977; Deacon & Henry, 1978) or cellulose film (Tribe, 1960, 1966; Griffith & Jones, 1963; Deacon, 1979) is open to criticism nevertheless, it might give a clue to what happens between cellulose decomposing species in the soil. However, what happens *in vitro* cannot signify what occur *in vivo* where pure substrates do not exist and fungi always present in large population and not in pairs or triads. Also, bearing in mind technique limitations and in addition that the soil is a complex environment inhabited by vast numbers of micro-organisms, our study is a preliminary one until such technique is available whereby a mixed population of organisms could be introduced at a time into a controlled soil system to imitate what is actually present in nature.

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